

LEGENDS TO SUPPLEMENTAL FIGURES

Figure S1

- (A) Schematic representation of the TAP-tagged Ph-p and Ph-d proteins. The TAP tag consisting of the protein A moiety (dark grey), the TEV protease cleavage site (thick black line) and the Calmodulin binding domain of Calmodulin binding protein (light grey) are indicated; FCS Zn-finger, Ph homology and SAM domains are labeled as in Fig. 1B.
- (B) Western blot analysis of nuclear extracts from *wild-type* (*wt*) and α -*tubulin1-TAP-Ph-p (TAP-Ph-p, left panel) or α -*tubulin1-TAP-Ph-d (TAP-Ph-d, right panel) transgenic embryos, probed with anti-Ph antibody. Expression levels of the TAP-Ph-p and TAP-Ph-d proteins cannot be directly compared to the levels of endogenous (endo) Ph-d and the two isoforms of Ph-p because on TAP-Ph proteins, the Ph antibody binds to both the Ph epitope and the protein A moiety.**
- (C) Cuticles preparations of *wildtype* (*wt*) embryos and embryos that were hemizygous for the *ph*⁵⁰⁴ null mutation that eliminates the function of both *ph-p* and *ph-d* (*ph*^{0 [mat+ zyg-]}); the mutant embryos carried no transgene (no TG) or a single copy of the α -*tubulin1-TAP-Ph-p (+ TAP-Ph-p) or the α -*tubulin1-TAP-Ph-d (+ TAP-Ph-d) transgene. *ph*^{0 [mat+ zyg-]} embryos show complete loss of ventral epidermis and incomplete dorsal closure; both phenotypes are fully rescued by the transgene-encoded TAP-Ph-p or TAP-Ph-d proteins. *ph*^{0 [mat+ zyg-]} embryos rescued by TAP-Ph-p or TAP-Ph-d also do not show the severe homeotic transformations of all thoracic and abdominal segments into copies of abdominal segment A8, except for the partial transformation of the A7 segment into A8; note also that head involution is defective and not fully rescued. All *ph*^{0 [mat+ zyg-]} animals carrying α -*tubulin1-TAP-Ph-p or α -*tubulin1-TAP-Ph-d arrested development at the end of embryogenesis.****
- (D) Scatterplot representation of the mass spectrometric data of the Ph-d-1 pull down. The ratio of label-free quantification (LFQ) intensities between the specific and the mock pull down is plotted versus the total protein intensity of the experiment. Interesting candidate proteins are highlighted in blue.

Figure S2

(A) Elution profiles of the Sfmbt-SAM:Scm-SAM^{L855E/L859E} complex compared with Sfmbt-SAM and Scm-SAM^{L855E/L859E} single domains run on the same SEC column (Superdex 75 16/60, GE Healthcare). Note the distinct shift of the peak of the complex ($V_{\text{Elution}}=69$ ml) containing the Sfmbt-SAM:Scm-SAM^{L855E/L859E} heterodimer (Fig. 2B) relative to the elution peaks that are observed when Sfmbt-SAM ($V_{\text{Elution}}=81$ ml) or Scm-SAM^{L855E/L859E} ($V_{\text{Elution}}=79$ ml) are expressed and purified as single domains. For simplicity, the Sfmbt-SAM:Scm-SAM^{L855E/L859E} complex is referred to as Sfmbt-SAM:Scm-SAM in the main text and figures.

(B) Coomassie stained SDS PAGE of GST-pulldown experiments comparing interaction of Scm-SAM versus Ph-SAM with GST-Sfmbt-SAM. Purified Scm-SAM or Ph-SAM were tested for binding to GST-Sfmbt-SAM (lanes 1-4) or, as control, to GST-GFP (lanes 5-8). Note that only Scm-SAM (compare lanes 3 and 1) but not Ph-SAM (compare lanes 4 and 2) interacts robustly with GST-Sfmbt-SAM. Asterisk mark degradation products. See Materials and methods for details.

(C) (Top) Elution profile of the Sfmbt-SAM:Scm-SAM:Ph-SAM^{L1561E/L1565E} complex run on the same SEC column (Superdex 75 16/60, GE Healthcare) as the complexes shown in (A). (Below) Coomassie stained SDS-PAGE of indicated peak fractions. Note the peak fraction contains stoichiometric amounts Sfmbt-SAM and the Scm-SAM:Ph-SAM^{L1561E/L1565E} fusion protein. Also note the shift of the elution peak ($V_{\text{Elution}}=61$ ml) relative to the elution peak of the Sfmbt-SAM:Scm-SAM^{L855E/L859E} dimer in (A).

Figure S3

Abd-B immunostainings of wild-type and Scm⁰ embryos without or with the indicated transgenes. (From left to right) Wild-type embryo, Scm⁰ embryo, Scm⁰ + F-Scm^{WT} and Scm⁰ + F-Scm^{ΔSAM}. Scm⁰ embryo without transgene shows deregulation of *Abd-B*. The F-Scm^{WT} transgene rescues repression of *Abd-B*. Embryos carrying the Scm^{ΔSAM} transgene are indistinguishable from Scm⁰ embryos.

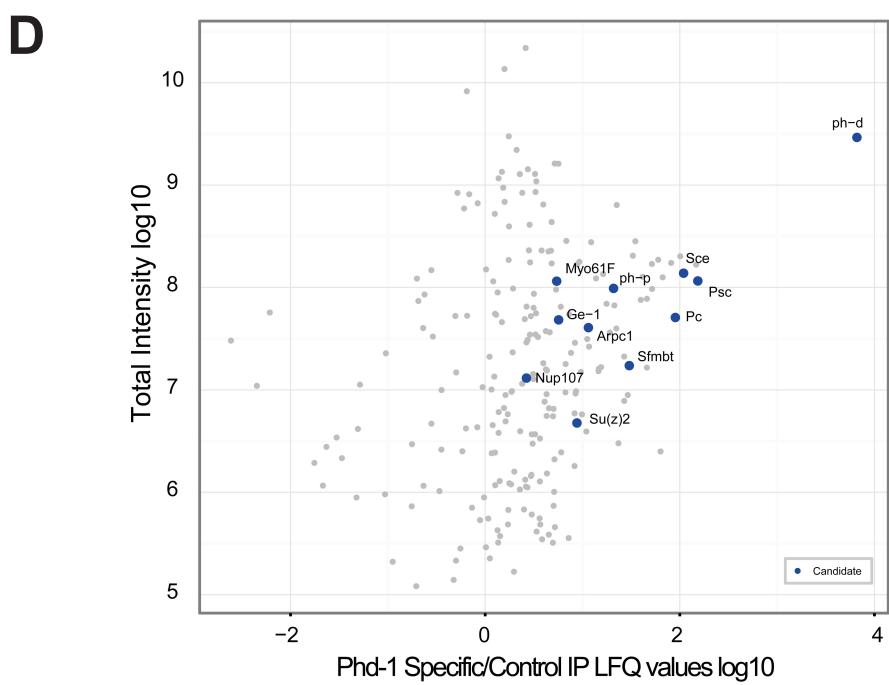
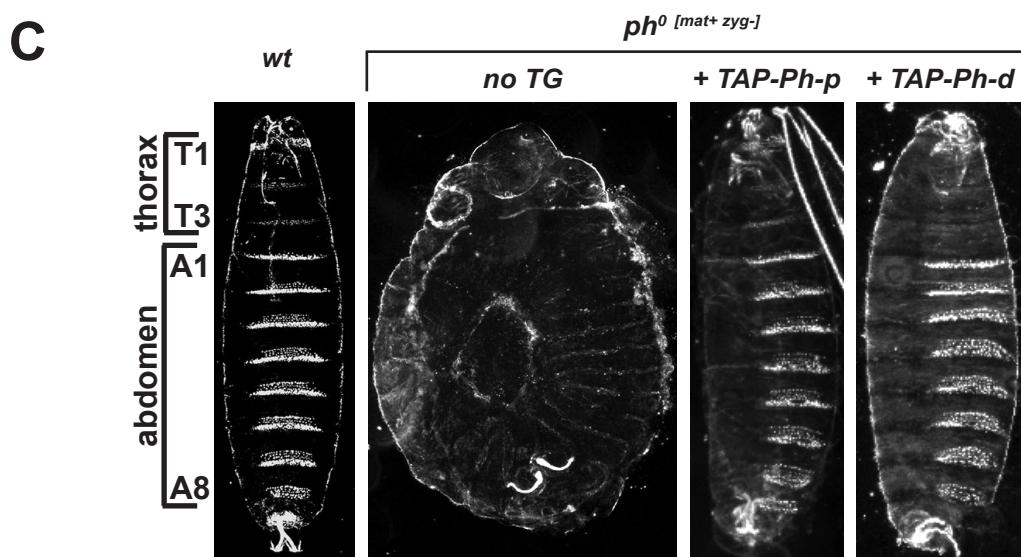
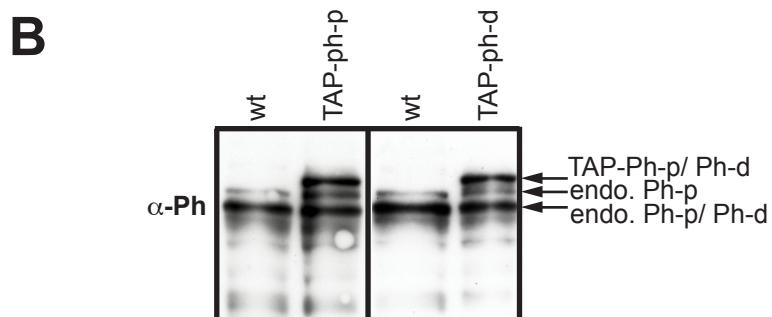
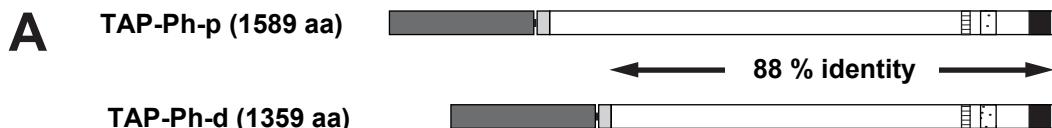
Figure S1

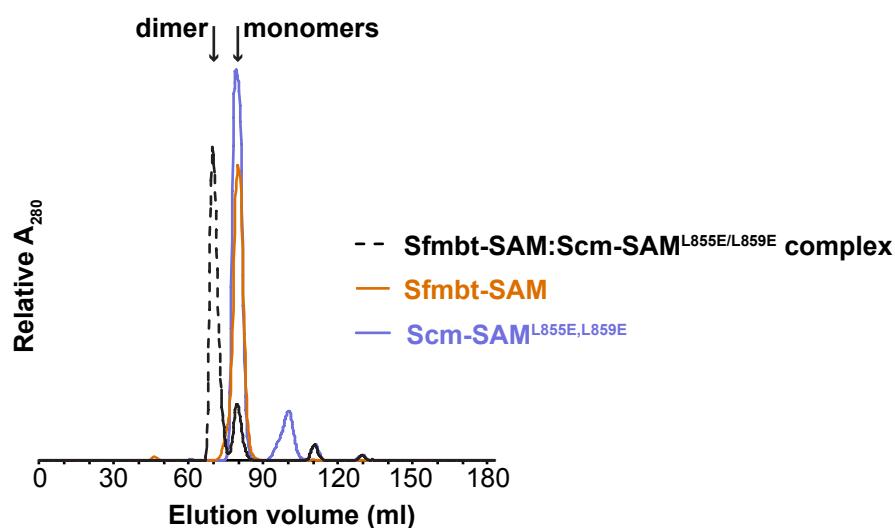
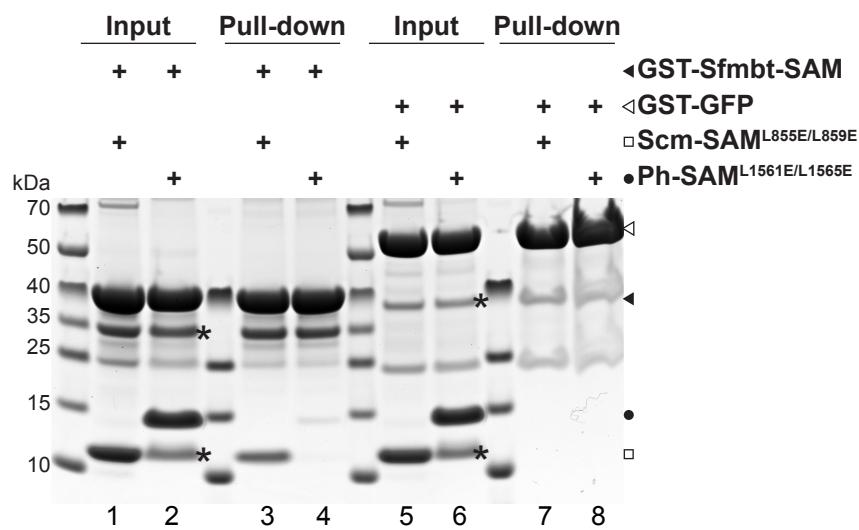
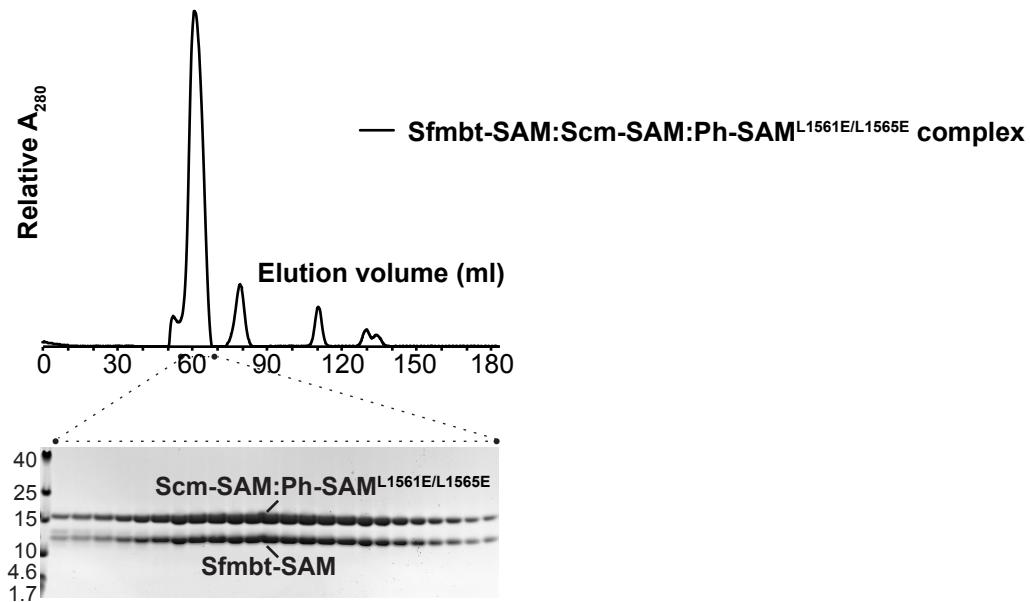
Figure S2**A****B****C**

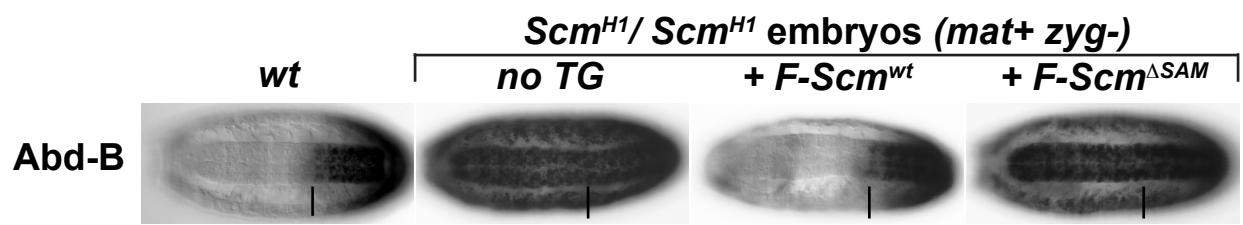
Figure S3

Table S1: Peptide sequences from mass spectrometry analysis of the proteins listed in Fig. S1D

Peptides identified in TAP of:	Ph-d 1	Ph-d 2	Ph-p
Ph-d (Polyhomeotic distal)			
AGISFDEDFAK	+	+	
ENHLVNAMEGMK	+	+	+
GPTATLVPIDSPK	+	+	+
GSVGTPSIR	+	+	
HPLPLPSSR	+	+	
QSNAAVQPPSSTIPNSVSGK	+	+	
QSNAAVQPPSSTIPNSVSGKEEPK	+		
RHPLLPLSSR	+	+	
TPPPSPEATTSVK	+	+	
VDPQRPLR	+	+	+
Ph-p (Polyhomeotic proximal)			
AGITFDEK			+
GPTATLVPIGSPK	+	+	+
HLVNAMGMK	+	+	+
HTSLTLEK		+	+
QSNAAVQPPSSTTPNSVSGKEEPK		+	+
QTHTPSTPNRPSAPSTPNTNCIAR			+
RADTESDTTPVSTTASQQISASAILAGGTPLK			+
YDVASPPHPGIAQQQATSGTGPATGSGVTPTSHR			+
Ph-d (Polyhomeotic distal)/ Ph-p (Polyhomeotic proximal)			
ATMQEDEK	+	+	+
CLETLAQK	+	+	+
ELPGCQDYVDDFIQQEIDGQALLLK	+		
LDEAMAEEK	+	+	+
LSGIASAPGSDMVACEQCQK	+	+	+
MVMSTTGTPITLQNQQLHAATAAGVDK	+	+	
NGIGGVGSGETNGLGGIVGVDAMALVDR	+		
NQPDGTQGMFIQQQPATQLQTQQNQIIQCNVTQPTK	+		
PASSVSTQTAQNQSLK	+	+	
PFQGNGPQMLTQQNAK	+		
PGAPVMPHNGTQVR	+		
QEFPHTTSGSGTELK	+	+	+
QQQLQLFQK	+	+	+
TEIGQVAGQNK	+	+	+
VGVDAQGK	+	+	+
VGVDAQGKLAQK	+		
VVGHLTTVQQQQATNLQQVVNAAGNK	+	+	+
YADKDVSDDEPK	+	+	+
Psc (Posterior sex combs)			
ASRPNPFAHIPNDVNR		+	
DRPEEAALATPEQR	+	+	+
DVAATPPTEALK	+	+	
EIVKPLKPEK		+	+
ENQEQQLAVEVASSK	+	+	
FEIDAQR		+	
FSIDIMYK	+		
FVYDKFEIDAQR	+	+	
IMSPSGVSTLSPR		+	
LPDQPQDQVQAQK	+	+	+
LSPLPLTVDFK		+	+
LTAAATAPQTK	+	+	+
LVPGLYER		+	+
QNSVTIIDMSDPERR		+	
QSPPAAVPGHLAAK	+		
RYQPILPK	+	+	
SDTTLQAIVYK	+	+	+
SIGGGSVENNNSNAAQKPHLYGPK	+		
SPSPLTVPPPLTIR	+	+	+

SPVNYYIEIVK	+	+	+
TAAGMQGSHSPTASENK		+	
TDSEPELVDTLRPR	+	+	
VATPPPPSSPR		+	
VEPVSLPEDQK	+	+	+
VEPVSLPEDQKAESIK	+		
VGNEVFNDYLQK	+		
VTPLKPVLTPTQVDK	+		
VTSGAFSEDPK	+	+	+
VYESPQPLVKPAPR		+	+
YLQCPAMCR		+	
Su(z) 2 (Suppressor of zeste 2)			
ILYDNEQTK	+	+	
INQDIEPEHNSVR	+		
LDSTSTSEALNR	+		
Sce/Ring (Sex comb extra)			
ADPNFDLLISK	+	+	+
AMSVLTSER		+	+
EEYEAIQEK	+	+	+
FCSDCIVTALR	+	+	+
FNQTQSQQALVNSINEGIK	+	+	
IYPSREEYEAIQEK	+	+	
KPQEVIDSTEIAVSPR	+	+	+
LTLDLGADLPEACR		+	+
MQVDDASNPPSVR	+	+	+
SEESESDSQMDCR		+	
SLHSELMCPICLDMLK	+		
STPSPVPSNSSSSKPK	+	+	+
TSLDPAPNK		+	+
TTANATVDHLSK	+	+	+
TWELSLYELQR	+		
Pc (Polycomb)			
DNATDDPVDLVYAAEK		+	
IASEAATQLK	+	+	+
LIDIYEQTNK	+	+	+
QPLTPLSPR		+	
VVITDVTVNLLETVTIR	+		
Sfmbt (Scm-related gene containing four mbt domains)			
DTGAVAAGQHLFHR	+	+	
GELYSLVLNTK	+	+	
GNIDPSVIPIQK		+	+
INDSLQSR		+	
ISDLIAQLK	+	+	
LVCVATVAR		+	
MNFTFDEYYSDGK		+	
VNDCTAHCDTSR	+		
Ge-1 (Enhancer of mRNA-decapping protein 4 homolog)			
IQNVAEFK		+	
IQPAALESGYLK	+	+	+
LMEQYLK	+	+	
LTEFLAAR	+	+	
MELLIDLVK		+	
NLSQLAYK		+	
QLHDAFSVGK		+	
QLLMAGQINK		+	
QLVESSLHK		+	
SIDS AVLQTIR	+	+	
SLEILLAR	+	+	
SQAATPAPPYDLR		+	
SSQFQCFQVK		+	
TELTDAMLETQR		+	
VCNIATSMR		+	
VLTELYR		+	+
VLTSGGVHTR		+	

Sop2 (Suppressor of profilin 2)/Arpc1

DIEEPPTPTPWGNR	+		
IFQSMDR	+	+	+
KPLGQLMAEFR	+	+	
LADVLNQHDLR	+	+	+
NAYVWTQGDDGK	+		
STVTSLDWHPNNVLLAGSTDYK	+		
TENTDTVVDSIHQNATSVR	+	+	
TQIALSPNNHEIHIYSR	+		
WKPALVLLR			+
Nup107 (Nucleoporin 107kD)			
ATAGVFSGHGSLK	+		
EVIQQLYALNATLR	+		
FLEQVEQK		+	+
GEQQNPLAHHDR		+	
IADASLELLNSK	+	+	
ILLQTDR	+	+	
IVAAVVEALIAR	+		
KPQTSHAASSQDNFTER		+	
LADEISSENRR	+	+	
LHEDPNFEQNVSVLHEK	+		
LPIEGNPR	+	+	
Myo61F (Myosin 61F)			
AGVQQLVK		+	
DKGDLILIPR		+	
DKGHLVIIGTQ		+	
DLVVTAAR		+	+
FILLSNK		+	
GDLILIPR		+	
GDVVTSPNQELAIYAR		+	
GGVIDIQTGAEPGVVR	+	+	
GHLVIIGTQ		+	
GIISILDEECLRPGEPTDK		+	
GQCVLISGESGSGK		+	
IDFTLTNHNDLDMVIR		+	
LLGVNASELEAALTHR	+	+	+
LNISLQAK		+	+
METGLHER		+	
NDAPNGFNEEFIANAK		+	
NNDLLFR		+	
NSLEHNVVK		+	
QVQQALTVIDFTK		+	
RPETAITQFR	+	+	
SLIEENR		+	
SNPVLEAFGNAK		+	
TLFDTEDAYQEK		+	+
TYELFLER		+	
VICNLIEEK	+	+	+
VNDFVASTFGSEQLK		+	
YLGLMENLR		+	

Table S2: X-Ray Data Collection and Refinement Statistics.

Data collection	
Beamline	SLS PXII
Space group	P1 21 1
Cell dimensions	
a, b, c (Å)	49.96, 53.97, 61.45
a, b, g (°)	90, 109.22, 90
Wavelength (Å)	1.0
Resolution range (Å)	47.17 – 1.975 (2.045-1.975)
Total reflections	193632 (12564)
Unique reflections	21180 (1797)
Completeness (%)	96.93 (83.18)
I/σI	11.21 (1.91)
CC (1/2)	99.8 (84.9)
R _{meas} (%) ^{a)}	14.5 (90)
Wilson B-factor (Å ²)	23.5
Refinement	
R _{work} (%)	25.5
R _{free} (%)	27.7
Ramachandran values	
Favored (%)	98
Allowed (%)	100
Rotamer outliers (%)	0
Overall average B factor (Å ²)	29.9
R.m.s.d bond lengths (Å)	0.017
R.m.s.d bond angles (°)	1.4

a) Diederichs K, Karplus PA. 1997. Improved R-factors for diffraction data analysis in macromolecular crystallography. *Nat Struct Biol* 4: 269-275.

Table S3: Drosophila strainsw¹¹⁸w ph⁵⁰⁴ FRT101/ FM7cyw; α -tubulin-TAP-Ph-d / TM6Cw; α -tubulin-TAP-Ph-p / TM6cScm^{H1} e/ TMB6 ubiGFPw; Flag-Scm^{wt}/ Cyo; Scm^{H1} e/ TMB6 ubiGFPw; Flag-Scm ^{Δ SAM}/ Cyo; Scm^{H1} e/ TMB6 ubiGFPScm^{H1} e/ TMB6 ubiGFPw; FRT2A FRT82B Scm^{H1} e/ TM6cw; Flag-Scm^{wt}/ Cyo; FRT2A FRT82B Scm^{H1} e/ TM6Bw; Flag-Scm ^{Δ SAM}/ Cyo; FRT2A FRT82B Scm^{H1} e/ TM6BSfmbt¹ FRT40/ Cyo ubi-GFPSfmbt¹ FRT40/ Cyo ubi-GFP; gSfmbt^{wt} [Vk33]/ TMB6Sfmbt¹ FRT40/ Cyo ubi-GFP; gSfmbt ^{Δ SAM} [Vk33]/ TMB6PRE_D line 5B (Fritsch et al., 1999)PRE_{D Pho mut} line 7.4 (Fritsch et al., 1999)

y w hs-Flp122; FRT82 hs-nGFP

y w hs-Flp122; hs-nGFP FRT40

Table S4: Antibodies

Specificity	Source/Reference
Abd-B	Developmental Studies Hybridoma Bank (DSHB) (1A2E9)
Ubx	DSHB (FP3.38)
Lamin	gift from D. Arndt-Jovin (T40)
E(z)	Gambetta et al, 2009
INO80	Klymenko et al, 2006
Caf1	Gambetta et al, 2009
OGT	Santa Cruz Biotechnology (sc-32921)
Pc	Papp and Müller, 2006
Ph	Oktaba et al, 2008
Pho	Papp and Müller, 2006
Psc	DSHB (6E8)
Scm	Gambetta et al, 2009
Sfmbt	Klymenko et al, 2006
Trx	Beisel et al, 2007
H3K27me3	Millipore (07-449)

References:

Beisel, C., Buness, A., Roustan-Espinosa, I.M., Koch, B., Schmitt, S., Haas, S.A., Hild, M., Katsuyama, T. and Paro, R. (2007). Comparing active and represed expression states of genes controlled by the Polycomb/Trithorax group proteins. Proc. Natl. Acad. Sci. 104, 16615-16620.

Table S5. Primers used for quantitative PCR (qPCR) to determine binding at specific chromosomal locations.

Name	to TSS	Forward primer (5' to 3')	Reverse primer (5' to 3')
PRE _D /PRE _D Pho mut	5' to PRE	GTGTCCAAATCCGTCGAATG	TTTAGTGGCCTTGCAGGTGA
PRE _D /PRE _D Pho mut	3' to PRE	CGTTTAAGTGCAGACTGAG	ATCAGCCGCCGATCCCTGGAG
Ubx	-31	CGCCAAACATTCAAGAGGATAG	GCAGCATAAACCGAAAGGA
Ubx	-30	CGTTTAAGTGCAGACTGAG	AAGGCAGAAAGAGAGCACCAA
Ubx	-29	TCCACCTCCTTCCCTCTC	GCACGCCTAAACCCATAA
<i>Abd-B</i>	+70	TAACCAGGGAGCAGCGACTT	GCCCCGAGATAAACCCGTTCT
<i>Abd-B</i>	+72	GCAGCCATCATGGATGTGAA	GGAATACCGCACTGTCGTAGG
<i>Abd-B</i>	+75	TACCGTTCACCGGCTCTG	GGGCAGTGGGAAGTCGTATTT
<i>CG14356 (Control1)</i>	-3	CCTAGGTGAATGTGCAGCACAC	ACACTGCGAGCGCCTCACACGC
<i>CG11665 (Control2)</i>	12	CAGTTGATGGATGAATTGG	TGCCTGTGGTTCTATCCAAAC